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Application of a Dried-DMSO rapid throughput 24-h equilibrium solubility in advancing discovery candidates

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ABSTRACT

A rapid throughput equilibrium solubility measurement is described. The method utilizes central liquid storage where compounds are stored as 10 mM solution in dimethyl sulfoxide (DMSO). The DMSO is subsequently removed to generate solid like material used for solubility measurement. A full range of available technologies is used including automated liquid handling, automated data collection using both HPLC/UV and LC/MS/MS. The method is fully validated and has been used to measure solubility for over 20,000 compounds across all phases of drug discovery. A detailed discussion on data interpretation and comparison to traditional solubility measurement using solid material is presented. An in-house solubility predictive model has been developed from the vast data set and has been employed successfully as part of compound design resulting in over 30% reduction in the number of poorly soluble compound synthesized.

1. Introduction

Physical properties, including solubility, lipophilicity, and ionization, are fundamental properties of potential drug candidates that govern their absorption, distribution, metabolism, and excretion (Lipinski et al., 1997; Avdeef, 2001; Gleeson, 2008). Among them, solubility is the most difficult property to predict and hence remains one of the most measured physical properties in a discovery organization. Advancing discovery candidates with good solubility and desired efficacy continues to be an industrial wide challenge (Lipinski, 2001). It has been estimated that poor water solubility could contribute up to 40% of attrition rate in drug development (Liu, 2008). Poor solubility has been related to poor data quality in *in vitro* assays (Weiss et al., 2002; Di and Kerns, 2006), variable exposure in *in vivo* observations (Zhou, 2008), and extensive involvement of formulation intervention (Huang and Dong, 2008). In light of high attrition rate in development due to toxicity and undesirable side effect profiles (Kola and Landis, 2004), recent publications have suggested more stringent physical properties, going beyond "Rule of Five" (Lipinski et al., 1997) for example using ClogP cutoff of 3.5, instead of 5 in drug design (Leeson and Springthorpe, 2007). The reduced ClogP would likely result in enhanced solubility (Jain and Yalkowsky, 2001).

Given the importance of solubility, its measurement has become a central component of a discovery organization. The practice of aqueous solubility measurement has evolved in the last decade due to increased demand and emergence of new technologies and instrumentation (Alsenz and Kansy, 2007). In the hands of many practitioners, starting the measurement using dry powder is generally preferred, as dry powder is thought to represent true thermodynamic state of the compound even though the solid-state in early discovery can be different from that in early development (Gardner et al., 2004). The main disadvantages using dry powder are difficulties in automating the process due to the lack of commercially available weighing robots that can weigh small amounts of diverse compounds into designated containers. The method also requires a minimum 2 mg of material, a relatively large amount of compound for those synthesized in early discovery. With the advancement of automated liquid handling, liquid storage facilities in support of High Throughput Screening (HTS), the methods used for solubility measurement since the 1990s have been able to draw upon central liquid storage dispensaries and their typical 10 mM DMSO stock solutions of drug candidates. The DMSO stock liquid is used to initiate solubility measurement and dispensed into aqueous buffer. The amount of DMSO is typically limited to $\leq$1% in the aqueous solution to minimize potential DMSO cosolvent effects; hence the determined solubility value has an upper limit of 100 μM. To obtain an upper limit of greater than 100 μM, one would need a higher stock concentration, sometimes difficult to achieve for poorly soluble compounds. A variety of approaches either kinetic

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or thermodynamic measurement have been used with different detection techniques including traditional HPLC-UV (Pan et al., 2001; Sugano et al., 2006), plate reader based systems such as nephelometers (Bevan and Lloyd, 2000) or ultraviolet (UV) absorption spectrometers (Colclough et al., 2008; Bard et al., 2008), and flow-through systems, either custom made (Lipinski et al., 1997) or based on flow cytometry (Goodwin et al). The wide spread availability of centrifugal evaporation in late 1990s has allowed DMSO removal under mild conditions and has facilitated another round of evolution in solubility measurement (Alsenz and Kansy, 2007; Zhou et al., 2007; Guo et al., 2008). The more recent method, we termed "Dried-DMSO" method, still rely on central compound DMSO stock storage for automated sample dispensary, but now DMSO is subsequently removed to generate solid like material prior to addition of buffer to initiate solubility measurement. Despite literature precedence and demonstrated success of the latter method, there are continued concerns about its utility beyond screening and early discovery. Here we wish to report our successful implementation of the method based on more than 8 years practice supporting more than 70 projects covering all phases of drug discovery from hit identification, lead identification, lead optimization and nomination for development. Our discussion includes specifics of the method, its validation, data interpretation and creation of an in-house solubility predictive model that has been shown instrumental in reducing the number of poorly soluble compound synthesized (Alelyunas, 2008).

2. Experimental

2.1. Instrumentation

LC/MS/MS was performed using Shimadzu pumps with a Micromass Ultima Triple Quadrupole mass spectrometer and a Leap Technology autosampler. HPLC/UV was performed using an Agilent 1100 (binary pump and multivariable wavelength UV detector) and Gilson 215 autosampler. ^{1}H NMR data were collected using Bruker 500 MHz NMR with Cryoprobe at 37 °C. Sample preparation and liquid handling were performed using a Tecan Genesis workstation. Dimethylsulfoxide (DMSO) was removed using a GeneVac HT4 centrifugal evaporation system. Sample mixing was performed using Eppendorf ThermomixerR fitted with a 96-well plate adaptor. Centrifugation was performed using an Eppendorf centrifuge Model 5810R. Atlas™ chromatography system was used in processing LC/UV data. Micromass QuanOptimize was used in acquiring and processing LC/MS/MS data. ActivityBase™ was used for polling LC/UV and LC/MS/MS data and entering data into AstraZeneca central database.

2.2. Chemicals and materials

All compounds mentioned in the present study were obtained from AstraZeneca in-house collection. HPLC grade acetonitrile was purchased from Fisher Scientific. Na_2HPO_4 and NaH_2PO_4 used for buffer preparation were obtained from Fisher Scientific. Inline filter and filter holder were purchased from Upchurch Scientific. The 96 glass vial well plates and 1.5-mL flat bottom glass vial inserts were obtained from Analytical Sales & Products Inc. Polypropylene 2 mL collection plate and plate mat were purchased from Phenomenex. StirStix (28 mm) and its 96-well dispenser were purchased from V&P Scientific Inc.

2.3. Sample preparation of the Dried-DMSO solubility method

A volume of 160 μL compound as 5 mM DMSO solution was dispensed into a 96-well Multitier plate by AstraZeneca central liquid dispensary. The plate was placed on a Tecan automation platform where 60 μL was withdrawn to make calibration and method development solutions for LC and LC/MS/MS. The initial plate containing the remaining 100 μL solution was then placed in GeneVac where DMSO was removed at 40 °C under full vacuum for 4 h. After drying, StirStix were added to the plate using a 96-well dispenser. The plate was then returned to Tecan workstation where 800 μL of pH 7.4, 0.1 M sodium phosphate buffer was dispensed. The plate was capped and the solution mixed at 750 rpm and 25 °C for 24 h. After mixing, the plate was centrifuged at 3000 rpm for 30 min. Using the Tecan, the middle portion of the liquid was transferred to two analytical plates for parallel LC and LC/MS/MS quantitation. Since more than 150 molar excess buffer concentration was used, the pH of the solution post mixing remained the same and was not measured on routine basis. An overview of the process is shown in Scheme 1. Each of the sample processing steps was extensively investigated, as detailed below. Typical throughput of the method is 1 plate per week using a fraction of a person's time. Since the system is modular, a higher throughput with multiple plates a day is possible. The potential bottlenecks are data acquisition and data processing. Details on plate mat choices and determination of residual DMSO by ^{1}H NMR and LC-UV are included in supplementary material.

2.4. GeneVac DMSO removal and sample recovery

Sample recovery using 44 randomly selected AstraZeneca compounds was determined. A Multitler plate containing 100 μL of test compounds was dried under full vacuum with both SampleGuard and Chamber temperature set at 40 °C and drying time of 4 h. After drying, 900 μL of DMSO was added to redissolve the compound. A reference solution was prepared by combining 100 μL of the test compound in DMSO stock and 800 μL DMSO. Sample recovery was calculated as the ratio of the HPLC/UV peak area of the dried sample versus reference solution. A maximum recovery of 100%, a minimum recovery of 80%, and an average recovery of 96 ± 6% were observed.

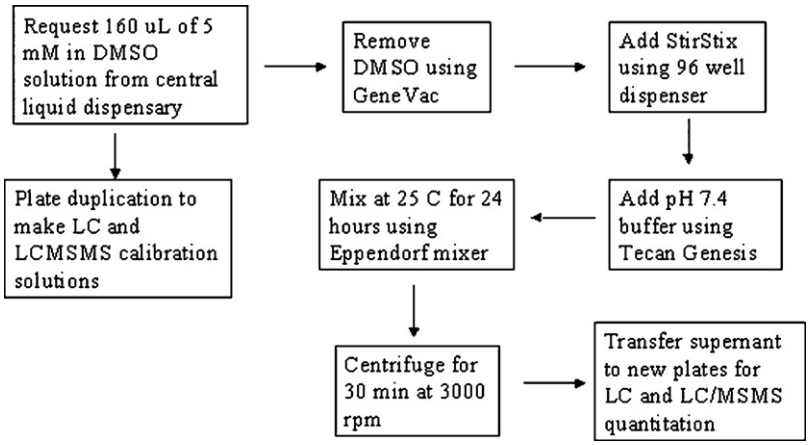
2.5. Effect of stirring on solubility measurement

The StirStix, a long hollow stainless steel tube coated with Teflon, used in the present study offered optimum mixing in a tall and narrow container. The vertical placement of the StirStix also helped in breaking up solid/film deposit formed at the bottom of the vial. The solubility of glyburide was determined with and without StirStix with all other aspects of sample handling and quantitation the same. The solubility of glyburide with varying amounts of DMSO up to 1% present was also determined with and without the StirStix. Other variables for the experiment were solution volume, shaking speed and different shakers. Data are presented in Table 1.

2.6. Sample recovery from filtration

2.6.1. Off-line filtration

Aqueous solutions of four poorly soluble AstraZeneca compounds with micromolar solubility were subjected to (1) centrifugation at 10,000 rpm for 30 min, (2) filtration using a 0.45 μm 3 mm stainless steel syringe filter (source: Upchurch Scientific), or (3) filtration using Whatman GFB 96-well filter plate. The filtrate or supernate was collected and quantified by HPLC-UV. Percent recovery was estimated assuming 100% recovery from centrifugation. Results showed the stainless steel filter performed the best overall for all four compounds. GFB filter plate resulted in 30% to complete loss of material (supplementary material). Unfortunately, the stainless steel filter is not available in 96-well format and thus not suitable for automation.



Scheme 1. Flow chart of the high throughput Dried-DMSO solubility measurement.

2.6.2. Inline filtration

The inline filtration was accomplished by installing an Upchurch Scientific inline filter unit between sample injection port and before the sample loop of the switching valve on the Gilson 215 autosampler. With each injection, sample passes through the filter first, prior to filling the sample loop. Three commercially available filters, PTFE, stainless steel, and titanium, were tested for potential absorptive sample loss. Tamoxifen and terfenadine dissolved in pH 7.4 phosphate buffer were quantified. Results indicated that stainless steel filtration gave the best overall performance and was chosen for use in the solubility measurements (supplementary material).

2.7. Preparation of sample plates for LC/UV and LC/MS/MS quantitation

Post-centrifugation, a Tecan Genesis liquid handler fitted with coaxial septum piercing tips was used to transfer samples to the analytical plate for quantitation. A nitrogen purging valve option was installed on the Tecan to control nitrogen on or off during sample transfer and to clean inside of the venting sleeve. For each sample transfer, the tips were inserted within 2 mm from the bottom of the vial where 550 μL liquids was withdrawn. The tips were then immersed in acetone and then water to remove potential solid adhering to the outside of the tip. A volume of 30 μL was discarded as waste followed by dispensing 340 μL into UV quantitation plate and 160 μL into MS/MS quantitation plate. A portion of Tecan program for valve control is included in supplementary material.

2.8. LC/UV quantitation

LC/UV quantitation was performed by passing the sample solution through a 2 μM stainless steel filter before filling the 10 μL sample 1oop. After sample injection, the autosampler rinses the inline filter by sequentially injecting acetone, acetonitrile, and ethanol. A generic gradient was used consisting of 5% ACN/0.1% TFA to 90% ACN/0.08% TFA in 4 min. The flow rate was 1.5 mL/min. Run time was 5 min. The column used was Halo (or Ascentis) C18 30 mm × 4.6 mm, 2.7 μm (ex Mac-Mod or Supelco). The detector wavelength was 220 nm. One standard solution containing 50 μM compound in 40% ACN/60% H_2O was used as the calibration solution for quantitation. The solubility is calculated according to the following:

$$\text{Solubility}(\mu\text{M}) = \frac{\text{Peak area of sample}}{\text{Peak area of standard}} \times \text{Concentration of standard}(50\,\mu\text{M})$$

2.9. LC/MS/MS quantitation

LC/MS/MS data were collected using a 5 μL sample loop and a Synergi Max RP 4 μ, 30 × 2 mm column. Data were acquired using Masslynx QuanOptimize™ software, which allowed automated method development, data acquisition and data quantitation. For MS/MS method development, the LC conditions used were based on a flow injection of a 5 μM solution in 40% ACN/60% H_2O at 0.2 mL/min flow rate and isocratic conditions of 50% 90:10 ACN:MeOH and 50% H_2O (0.1% formic acid). For sample quantitation, a generic gradient was used consisting of 100% H_2O (0.1% formic acid) to 95% 90:10 ACN:MeOH in 1.6 min at 1.5 mL/min flow rate and 2 min run time. The post column split ratio is 1:50. The concentration of calibration solution was 1 μM. The solubility is calculated according to the following:

Table 1
Summary of stirring effect on glyburide solubility at pH 7.4.

Entry	Method	StirStix present	% DMSO in solution	Measured solubility (μM)[a]	Solution volume (μL)	Shaking speed (rpm)	Amount of compound added (μM)
1	Solid	Yes	0	6–14	Various	Various	Various
2	Dried-DMSO	Yes	0	6–12	800	750	500
3	1% DMSO	No	1	60–100	Various	Various	100
4	1% DMSO	Yes	0.25–1	6–10	800	750	100
5	Dried-DMSO	Yes	0.25–1	6–12	800	750	500
6	Dried-DMSO	No	0	410 (9)	800	750	500
7	1% DMSO	No	1	82 (2)	800	750	100
8	1% DMSO	No	1	84 (2)	800	350	100
9	1% DMSO	No	1	87 (3)	400	350	100
10	1% DMSO	No	1	79 (1)	800	~350	100
11	1% DMSO	No	1	83 (3)	400	~350	100

[a] Data in parenthesis is standard deviation based on 8 measurements.

$$\text{Solubility}(\mu\text{M}) = \frac{\text{Peak area of sample}}{\text{Peak area of standard}} \times \text{Concentration of standard}(1\ \mu\text{M})$$

2.10. Data reporting

The LC/UV and LC/MS/MS data were imported into an ActivityBase worksheet for solubility calculations and data entry into AstraZeneca central database. For compounds with solubility >10 μM, LC/UV data were reported. For compounds with solubility <1 μM, LC/MS/MS data were reported. For the intermediate concentration range of 1–10 μM, the higher value of the two techniques was reported. The upper limit of data reported is 500 μM which is less than the sample loading of >600 μM (100 μL stock solution × 5 mM stock concentration/800 μL buffer volume) to compensate for potential sample loss during GeneVac evaporation process. Sample purity information based on the percent parent of all peaks observed in the 50 μM calibration solution at the 220 nm was also reported.

2.11. Quality control

Glyburide was run with each batch of solubility measurement and used as the quality control compound. A glyburide DMSO stock solution was prepared, stored, and dispensed by the AstraZeneca central liquid dispensary in exactly the same manner as all other test compounds. Data collected from 134 individual measurements spanning over 3 years indicate good reproducibility, 10.0 ± 2.6 μM at pH 7.4.

2.12. Solid method

The solid solubility method refers to solubility determination using dry powder as the starting material. For the measurement, 1 mg dry powder was weighed into the 1.5 mL Multitier glass vial directly followed by addition of StirStix and 800 μL buffer. The liquid handling, sample transfer, and quantitation were the same as the Dried-DMSO method. Because a large amount of solid was used compared to the Dried-DMSO process, the solution after shaking was centrifuged twice at 3000 rpm for 30 min before transferring to an analytical plate for quantitation.

2.13. Historical kinetic and equilibrium solubility measurement using solid as the starting material

Data presented in Fig. 1 were collected by dispensing 1 mg dry powder into a 1.5 mL microcentrifuge tube with stirrer bar and 1 mL pH 7.4 phosphate buffer. After stirring for 1 h, 500 μL of the solution was transferred to a new microcentrifuge tube and centrifuged at 12,000 rpm for 30 min. The supernatant was transferred into an autosampler vial for HPLC/UV quantitation. After stirring the remaining solution for another 23 h, the solution was centrifuged; sample transferred and analyzed the same way as the 1 h sample.

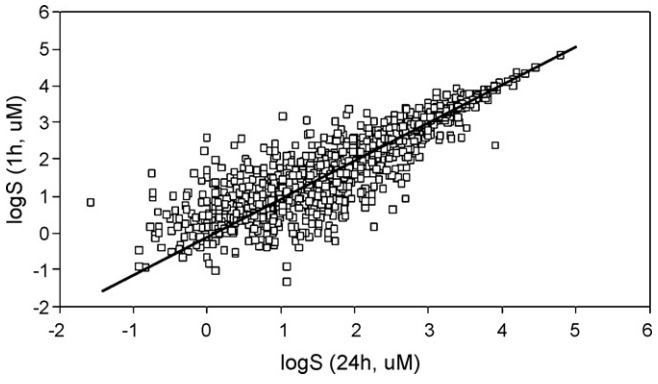


Fig. 1. Solubility plot of 1 h solubility versus 24 h solubility. The line drawn is orthogonal line fit.

2.14. Solubility predictive model

The model was developed using support vector machines with a radial kernel function and nu-regression as implemented in the R-Project (RDCT, 2007). Molecular descriptors used include the AlogP of Ghose et al. (1998), molecular weight, numbers of hydrogen bond donors and acceptors, number of rotatable bonds, number of rings, number aromatic rings, polar surface area as calculated by Ertl's method (Ertl et al., 2000), and 128 SciTegic FCFP_4 fingerprint features (Pipeline, 2004). pK_a might be a good descriptor for solubility prediction at specific pH. However, it is not used as a descriptor in our silico model as accurate pK_a prediction itself is a very challenging task. Over ten thousand compounds with numerical solubility data measured by the Dried-DMSO method were available in 2005. The compounds were randomly separated into a 70% training set (7176 compounds) and a 30% test set (3730 compounds). The final model derived has a root mean square error (RMSE) of 0.55 log unit and a squared correlation coefficient of 0.81 for the training set compounds. The RMSE and squared correlation coefficient for the test set compounds are 0.75 and 0.64, respectively.

Since the solubility data we used to build the in silico model were measured in pH 7.4 buffer, the QSAR model is an apparent solubility model. The model predictions for acidic and basic compounds should not be directly compared to intrinsic solubility. Some of our project teams used to use Accelrys' aqueous solubility prediction model (Cheng and Merz, 2003) to prioritize synthesis candidates. It was observed that the Accelrys' model predictions are significantly lower than our measured values for basic compounds. Upon careful examination, we realized that the Accelrys model was derived from solubility in un-buffered solutions. Because basic compounds ionize to a much smaller degree in un-buffered solutions than in pH 7.4 buffer, it is understandable that the model predictions are much lower than apparent solubility measured at a fixed pH of 7.4. This underlines the difficulty of comparing performance of our prediction model to those commercially available, as commercial models are trained with solubility data from different sources measured under different conditions.

3. Results and discussion

3.1. General description of the Dried-DMSO equilibrium solubility method

A rapid throughput Dried-DMSO equilibrium method has been developed and used as the primary method for solubility profiling of discovery drug candidates. The method utilizes DMSO stock stored in the central liquid dispensary where the solution is prepared automatically upon new compound submission. By removing DMSO and generating solid material, the method minimizes concern of potential DMSO effect on measured solubility (Lipinski, 2008). The buffer pH is 7.4, instead of 6.8 commonly used in solubility measurement for mimicking gastrointestinal pH (Dressman et al., 1998). Since this laboratory is focused on CNS discovery, pH 7.4 is used to parallel the pH of systemic circulation; and there is no pH gradient across blood–brain barrier. Available in-house data also suggest that the pH 7.4 solubility value can be relevant to *in vitro* ADMET assays since the pH of most of the assays are 7.4.

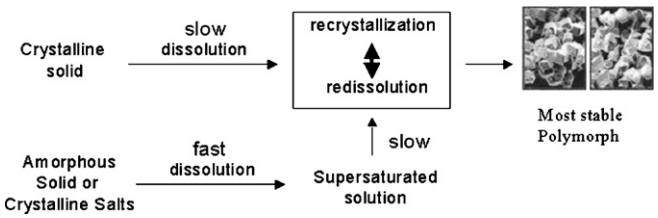
The sample preparation is fully automated with minimal manual intervention involving moving the plate from one instrument to another. For sample workup prior to quantitation, the method uses both centrifugation and inline filtration to minimize potential sample loss compared to use of only off-line filtration. By employing both LC and LC/MSMS for quantitation, the determined solubility covers full solubility range from nM to >500 μM. The lower limit depends on compound's sensitivity under MS/MS ionization conditions. A single calibration point was used in both UV and MSMS quantitation to maximize throughput. The LC-UV run time is scheduled to allow maximum gradient spread to separate impurities from the parent and also ensure completion of 96 compounds in one overnight run. Each step of the method has been extensively validated as discussed in the following sections. Data interpretation of the Dried-DMSO solubility is discussed based on detailed data analysis of project compounds collected in 2007.

3.2. Effect of stirring on measured solubility

Our observations in the present study suggest that stirring is one of the most important experimental variables in solubility determination. The need for vigorous stirring is often stressed in solubility standard methods and review literature, but in practice the specifics of stirring are subject to individual interpretation. The method of stirring is not always detailed, for example the ASTM method (Standard Test, 1993). The lack of a stir bar or like used in some recent literature may be due to the fact that adding a stir bar is not an automated process per se. The most efficient way may be the use of a 96-well dispenser such as the one used in the present method. Early indication of the importance of stirring on measured solubility came from attempts to understand glyburide solubility data collected across AstraZeneca as part of a cross site, cross method comparison exercise. Initial data indicated large differences with glyburide solubility much higher when 1% DMSO was present than using solid/Dried-DMSO methods (entry 1–3, Table 1). Note that for all three methods, the 24 h stirring time was the same and quantitation (LC-UV) similar. But when glyburide solubility was measured in the presence of 0.25 to 1% DMSO using our standard setup, the value obtained is essentially the same as Dried-DMSO method (entry 4, 5). Upon inquiry, we learned that a stir bar was not used in the original 1% DMSO data. Using our current method, the next set of experiments was carried out using the same settings with the exception that StirStix was not added. Indeed, the higher value was reproduced (entry 7 and 3, Table 1). Interestingly, the glyburide solubility in the absence of StirStix was highly reproducible and independent of solution volume (compare entry 8 and 9, and 10 and 11), shaking speed (compare entry 7 and 8), and differences in the shaker used (compare entry 8 and 10, and 9 and 11). When higher amount of glyburide was introduced, a higher solubility was also observed in the absence of StirStix (compare entry 7 and 2). These data suggest to us that glyburide is capable of forming and maintaining supersaturated solutions for an extended time. The stirring decreased its solubility. The phenomenon can also be visualized, e.g. solutions without StirStix remained transparent after 24 h mixing. When StirStix was present, the solution turned cloudy with white precipitate within minutes of mixing.

3.3. Sample drying under GeneVac conditions

Using the Dried-DMSO method, DMSO was removed to form solid material prior to making solubility measurements. The amount of residual DMSO was estimated by ^{1}H NMR to be <0.005% DMSO. Visual inspection after drying indicated some compounds formed dry powder. The majority compounds appeared as dry film, which is indicative of amorphous material (Yalkowsky, 1999).



Scheme 2. A simplified scheme for mechanism of solubilization.

Visual inspection of precipitates formed after 24 h mixing in buffer indicated the presence of crystalline or semi crystalline material for some compounds, for other compounds the precipitates appeared to be waxy material adhering to the side of the vial. Under GeneVac drying conditions, evaporative loss was observed for some low molecular weight compounds resulting in solubility underestimation.

3.4. Discussion on mechanism of solubilization

Data interpretation of the Dried-DMSO versus solid solubility needs to be explained in the context of mechanism of solubilization. For the purpose of the present discussion, the mechanism of solubilization is broadly divided into two major phases: a dissolution phase and a recrystallization/reequilibration phase (Scheme 2). The discussion of solid-states are limited to crystalline solid, amorphous solid, and salt crystalline solid. For a more detailed discussion, readers are referred to many excellent publications (Bhugra et al., 2008; Yalkowsky, 1999). For a solubilization to occur, a compound suspended in an aqueous media will go through a dissolution phase followed by recrystallization/reequilibration to form the most stable polymorph. The rate-limiting or the slowest step in the process would depend on dissolution behavior of the solid. For crystalline solid, the dissolution step is likely to be the rate-limiting step. For amorphous solid and salts known to have fast dissolution, the rate-limiting step is likely to be precipitation/recrystallization from the initial supersaturated solution followed by reequilibration to form the most stable polymorph. For example, in the absence of a stir bar, data presented in the previous section suggests that glyburide can stay as a supersaturated solution at >400 μM for over 24 h and at a shaking speed of 750 rpm when the compound equilibrium solubility is rather low ~10 μM. The potential effect of different solid forms on measured solubility will be dependent on whether equilibrium is reached with the set 24 h stirring. When the most stable polymorph having the lowest solubility (Bhattachar et al., 2006) is formed, solubility should be independent of the starting solid-state, whether it is crystalline, amorphous, or salts. On the other hand, when equilibrium solubility is not reached within 24 h, one would likely see different solubility with amorphous solid and salts offering higher solubility (Gruta et al., 2004).

3.5. Compound equilibration at 24 h

The 24 h stirring time is a commonly employed time point for equilibrium solubility measurement. The question remains that if this 24 h timeframe is sufficient for a compound reaching equilibrium. To answer this question, a data set collected by our group in 1997–1998 was examined. The solubility measurement carried out at that time was based on starting with dry powder and sampling at both 1 h and 24 h. The data were collected for about 2200 compounds covering diverse projects. A plot of 1 h versus 24 h solubility is shown in Fig. 1. Using 3-fold difference as the cutoff criteria, the data suggest that for 77% of the compounds, the 1 h solubility is the same as 24 h solubility, that is, for these compounds the equilibrium is likely reached within 1 h of stirring. There are 12% of

Table 2
Summary of Dried-DMSO and solid solubility of 31 commercially available compounds in pH 7.4, 0.1 M sodium phosphate buffer at 25 °C.

Entry	Compound name	MW	ClogP	Ion class[a]	Dried-DMSO solubility (μM)	Solid solubility (μM)	Ratio (Dried-DMSO/solid)	Lit. solid solubility (uM)	Lit. method	Lit. Reference
1	Albendazole	265.3	3.46	Neutral	1.0	0.77	1.3	2.8	pH 7.4, 37 °C, 1 week	(Daniel-Mwambete et al., 2004)
2	Amiodarone	645.3	8.95	Base	0.01	0.0048	1.9	0.015	pH 7, 37 °C, 24 h	(Glomme et al., 2005)
3	Anagrelide	256.1	1.02	Base	5.8	4.7	1.2	4.7	pH 4–8, 25 °C	(Burnside et al., 2004)
4	Astemizole	458.6	5.84	Base	2.9	3.1	0.9			
5	Bifonazole	310.4	4.74	Base	0.36	0.3	1.2	1.6	pH 8–10, 25 °C, 2 h	(Popovic and Cakar, 2004)
6	Bitolterol	461.6	5.59	Base	32	105	0.3			
7	Chlorpromazine	318.9	5.3	Base	446	>500	0.9			
8	Cinnarizine	368.5	6.05	Base	0.37	0.25	1.5	5.4	pH 7.2	(Parikh et al., 2006)
9	Clofazimine	473.4	7.70	Neutral	0.21	0.15	1.4	0.48	H_2O	(Data, 2009)
10	diethylstilbestrol	268.4	4.96	Neutral	25	19	1.3	23.1	pH 6.8, 22 °C, 24 h	(Zhou et al., 2007)
11	Diphentoin	252.3	2.08	Neutral	83	81	1.0	127	H_2O	(Data, 2009)
12	Disulfiram	296.5	3.88	Neutral	37	7.3	5.0	13.8	H_2O	(Data, 2009)
13	Estrone	270.4	3.38	Neutral	2.1	2.2	1.0	4.8	H_2O, 25 °C, 4 days	(Shareef et al., 2006)
14	Felodipine	384.3	5.30	Neutral	1.1	0.64	1.7	2.2	pH 7.0, 37 °C, 24 h	(Glomme et al., 2005)
15	Fenofibrate	360.8	5.23	Neutral	0.73	0.21	3.5	2.2	H_2O, 25 °C, 15 h	(Jamzad and Fassihi, 2006)
16	Flutamide	276.2	3.33	Neutral	87	79	1.1	72.4	pH 7.4, 24 h	(Holler and Valenta, 2007)
17	Glyburide	494	4.24	Acid	10.0	8.9	1.1	11.4	pH 7.0, 37 °C, 24 h	(Glomme et al., 2005)
18	Griseofulvin	352.8	2.05	Neutral	17	15	1.1	13	H_2O, 22.5 °C, 24 h	(Bergstrom et al., 2002)
19	Haloperidol	375.9	3.85	Base	52	64	0.8	~27	pH 7.4, 37 °C, 24 h[b]	(Li et al., 2005)
20	Ketoconazole	531.4	3.63	Base	14	2.9	4.6	11.3	pH 7, 37 °C, 24 h	(Glomme et al., 2005)
21	Loperamide	477	4.66	Base	43	3.0	14.1			
22	Loratadine	382.9	5.05	Neutral	2.0	1.5	1.3	8.7	pH 7.5, 25 °C 24 h	(Popovic et al., 2009)
23	Mebendazole	295.3	3.08	Neutral	1.0	0.29	3.5	3.7	pH 7.4, 37 °C, 1 week	(Daniel-Mwambete et al., 2004)
24	Moricizine	427.5	2.64	Base	66	47	1.4	42.1	pH 7, 25 °C, 48 h	(Hussain et al., 1993)
25	Nifedipine	346.3	3.12	Neutral	166	98	1.7	12.8	pH 7 tris buffer, 30 °C, 6 h	(Devarakonda et al., 2004)
26	Nimodipine	418.4	4.00	Neutral	5.0	1.6	3.1	7.3	pH 7, 37 °C, 48 h	(Yu et al., 2006)
27	Phenytoin	252.3	2.08	Neutral	86	81	1.1	81.2	pH 7.4. 25 °C, 24 h	(Schwartz et al., 1977)
28	Tamoxifen	371.5	6.82	Base	0.82	1.2	0.7	0.1	H_2O, 37 °C, 24 h	(Gao and Singh, 1998)
29	Terfenadine	471.7	6.07	Base	4.1	2.9	1.4	3.8	pH 7, 37 °C, 24 h	(Glomme et al., 2005)
30	Testosterone	344.5	4.88	Neutral	63	55	1.1	76.2	H_2O, 22.5 °C, 24 h	(Bergstrom et al., 2002)
31	Verapamil	454.6	4.47	Base	465	>500	0.9	1584	pH 7.4, 22.5 °C, 24 h	(Bergstrom et al., 2004)

[a] Refer to ionization at neutral pH.
[b] Approximate value, estimated from pH–solubility graph mentioned in the reference.

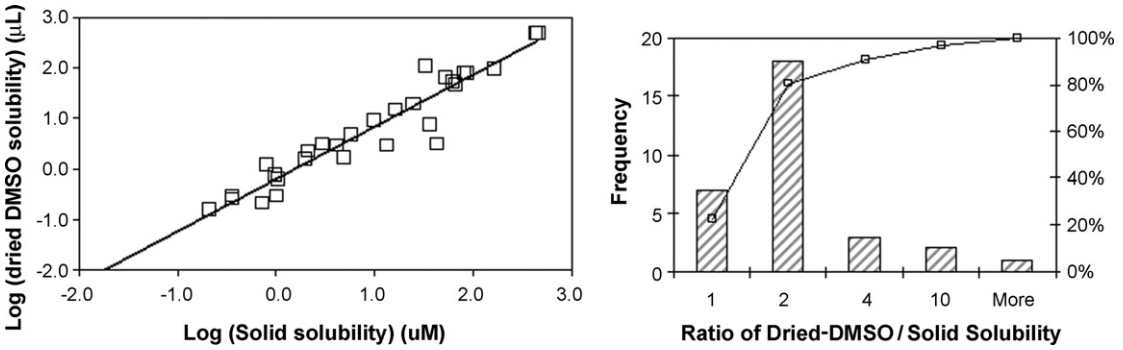


Fig. 2. Correlation of Dried-DMSO solubility versus solid solubility. Left: plot of log (Dried-DMSO) solubility versus log (solid solubility). Right: Histogram of the ratio of Dried-DMSO/solid solubility.

compounds that 1 h solubility is lower than 24 h, suggesting solubility for these compounds are dissolution limited. There are 11% of compounds that 1 h solubility is higher than 24 h, suggesting solubility for these compounds are precipitation/recrystallization limited. The more soluble compounds also showed less deviation than more poorly soluble compounds. In summary, the result indicates that for the majority of compounds the equilibrium is reached with 24 h stirring, consistent with literature observations (Guo et al., 2008; Chen and Venkatesh, 2004).

3.6. Correlation of Dried-DMSO solubility with solid solubility of known drugs

Solubility was determined for 31 commercially available compounds with differing ionization states using both Dried-DMSO and solid method. Data are summarized in Table 2 and plotted in Fig. 2. A good agreement was observed for greater than 80% of compounds with the ratio of Dried-DMSO over solid within three times experimental reproducibility. This good agreement indicated for these compounds, equilibrium has been reached with 24 h mixing. Large differences were observed for a few compounds with Dried-DMSO value typically higher than the solid value. As discussed earlier, the large differences suggest that longer than 24 h stirring time is needed to obtain equilibrium solubility especially when starting with dry powder. Where available, literature solid solubility values determined under comparable conditions are included in Table 2. Since solubility value could be affected by many variables, literature experimental conditions used for the measurement such as solubility media, temperature and time duration of mixing are also listed. A good agreement was observed for greater than 75% compounds between literature and solid value measured in the present study.

3.7. Reproducibility of the Dried-DMSO solubility method

Data reproducibility of the Dried-DMSO method was analyzed based on: (a) reproducibility of quality control compound whose solubility was measured over time and (b) reproducibility of project compound of the same batch when the measurement was repeated at a different time. Glyburide is used as the quality control compound and is included in each set of solubility determination, typically run on a weekly basis. Data collected over ~3 years span indicated good reproducibility with glyburide solubility 10.0 ± 2.6 μM at pH 7.4 with 2% RSD. Good reproducibility was also obtained in repeated measurement for the same batch of project compounds with % RSD less than 50% for 91% compounds (Fig. 3). Because of the observed good reproducibility, the solubility of each compound is measured as singleton to enhance throughput.

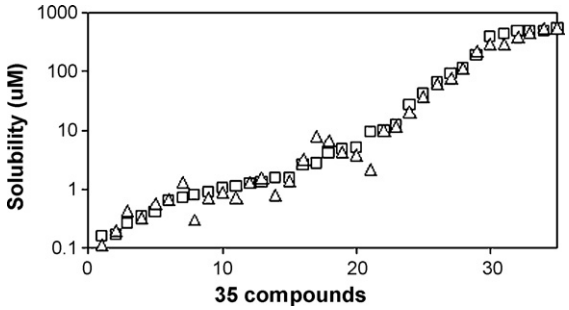


Fig. 3. Reproducibility of solubility measurement performed in 2007 for the same batch of compound. (□) Initial data and (△) repeated data for the same batch.

3.8. Dried-DMSO solubility of compound with multiple batches

When a project progresses from lead identification to lead optimization, promising compounds will be synthesized in multiple batches and registered as such. When a salt is made, it is also given a new batch number. In our solubility strategy, solubility value is determined automatically for all newly registered compounds up to batch five. In 2007, there were 79 compounds for which solubility was measured for multiple batches, most of them had two batches and a few had up to 5 batches. Data summarized in Fig. 4 indicated generally good reproducibility. Considering potential difference in crystallinity and salt forms of the batches, the good reproducibility suggests potential solid-state normalization using the Dried-DMSO process since different batches of dry powder are dissolved and dried in the same manner. This normalization and potential of providing consistent solubility value for a given compound at different phases of discovery can be an added advantage of Dried-DMSO method compared to solid method.

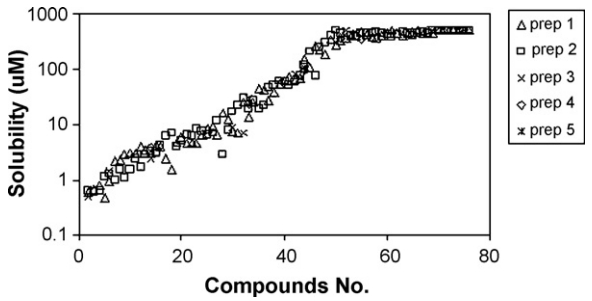


Fig. 4. Measured solubility for multiple batches of the same compound. For visualization purposes, compounds are ordered based on their solubility from low to high.

3.9. Effect of impurity on measured solubility

Impurities present in discovery compound could affect the accuracy of measured solubility. In principle, impurity can affect solubility in one of two ways (a) by altering concentration of the calibration solution. For example, a compound is calculated to have 100 μM solubility assuming 100% purity. If the compound is only 85% pure, the solubility after purity correction becomes 85 μM, that is, the original reported solubility is an overestimate, and (b) by affecting precipitation from supersaturated solution. The latter effect is expected to be more pronounced using Dried-DMSO method compared with solid method. In both cases, the impurity would likely result in an overestimation of solubility. To minimize potential error in measured solubility, a cutoff value of 85% purity is used to report data. When % purity is less than 85%, solubility is reported as "NV" (no value).

3.10. Effect of chiral impurity on measured solubility

When a compound containing chiral centers is synthesized, it is registered with different identification numbers to differentiate whether it is a single isomer, or racemic mixture/diastereomer. Compounds registered as a single isomer could contain trace amount of the opposite isomer and the amount of chiral impurity is not usually recorded in the registration. Unlike impurities mentioned above, chiral impurity is difficult to detect under the generic non-chiral LC separation conditions. While diastereomer could have different solubilities, the solubility of enantiomers is expected to be the same. Data presented in this section suggest that chiral impurities can potentially have larger effect on Dried-DMSO solubility than the solid solubility. Our initial awareness of the effect came from analyzing data collected for AZ01. Dried-DMSO solubility value of AZ01 indicated a large decrease from 240 μM in prep 1 to 28 μM in prep 3 while the solid solubility remained essentially the same (entry 1, Table 3). There was an apparent convergence in prep 3 where solubility value from both methods became essentially the same. Upon consulting the synthetic chemist responsible for its synthesis, we learned that prep 1 contained ~10% chiral impurity and prep 3 was the purest batch (with >99% purity). To further explore the potential chiral impurity effects, chiral compounds were extracted from 2007 data. In 2007, there were 99 sets of chiral compounds; 42 sets contained single chiral center or enantiomers, 57 sets contained two or more chiral centers, a mixture of diastereomer and enantiomers (Fig. 5). Of the 42 enantiomer sets, the Dried-DMSO solubility were essentially the same for 35 sets. Two compounds showed different solubility of each enantiomers. The five remaining compounds showed similar solubility for each enantiomer, but much higher solubility of racemic mixture (Table 3, entry 2–6). The differences were much larger than the batch-to-batch variation shown in Fig. 4. In the case of diastereomer, two compounds, where one chiral center is fixed and the second chiral center is 1:1 mixture, the Dried-DMSO value of the mixture is also higher than individual diastereomer (Table 3, entry 7–8). When solid solubility was measured for one of the diastereomer sets, the solid value of the mixture is lower than the Dried-DMSO

Table 3
Solubility summary of project compounds.

Entry	Compound	Prep	Dried-DMSO (μM)	Solid (μM)	Notes
1	AZ01	Batch 1	236	23	Contain 10% chiral purity
		Batch 2	79	51, 32	
		Batch 3	28	22, 23	>99% ee
2	AZ02	Mixture	17		
		Isomer1	3.01		
		Isomer2	3.12		
3	AZ03	Mixture	132		
		Isomer1	24.4		
		Isomer2	41.6		
4	AZ04	Mixture	14.9		
		Isomer1	5.56		
		Isomer2	5.58		
5	AZ05	Mixture	42.4		
		Isomer1	22.6		
		Isomer2	26.5		
6	AZ06	Mixture	36.5		
		Isomer1	12.9		
		Isomer2	28.1		
7	AZ07	Mixture	96.2	15.1	
		Isomer1	5.71	6.1	
		Isomer2	3.41		
8	AZ08	Mixture	31.8		
		Isomer1	14.6		
		Isomer2	11.2		
9	AZ09	Batch 1	0.61		
		Batch 2	0.5		Sulfuric acid
10	AZ10	Batch 1	333		Purity = 86%
		Batch 2	194, 295	198	Citric salt
		Batch 3	219, 211	79	Free-base
11	AZ11		95	5.2	Hydrate form
12	AZ12	Batch 1	279		
		Batch 2	170		
		Batch 3	184, 178	87	Stable polymorph
		Batch 4	189, 134	14	Stable polymorph
		Batch 5	185		
13	AZ13	Batch 5	14	3.5	Crystalline
		Batch 6		15	Lyophilized
		Batch 7		12	Lyophilized

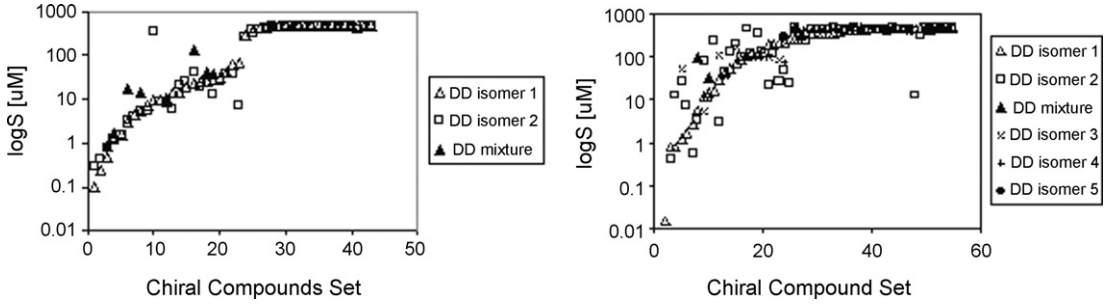


Fig. 5. 2007 solubility of chiral compounds, plot of compound versus Dried-DMSO (DD) solubility (μM). (Left) having one chiral center, and (right) having two or more chiral centers.

value, while the solid value of the single isomer is the same (Table 3, entry 7). This observed potential higher solubility using the Dried-DMSO method in the presence of chiral impurities might be similar to observations in recrystallization where single crystal is known more difficult to form from solutions containing the opposite isomer. Because of this potential solubility overestimation using the Dried-DMSO method, projects are recommended to request solid solubility when large differences between batches and isomers are observed.

3.11. Effect of salts on measured solubility

A common practice to enhance dissolution rate of poorly soluble compounds is to convert the compound from salt-free to salts. The salt form is thought to increase solvation and thus enhance dissolution, important for *in vivo* sample preparations. Under conditions used in the present solubility measurement, the compound is stirred in >150 molar excess phosphate buffer at pH 7.4. The initial salt form is likely converted into either phosphate salt or salt-free, based on the compound's pK_a value. The actual effect would depend on whether the most stable polymorph is reached with 24 h mixing. When it is reached, the salt effect is expected to be minimal. But when it is not reached and when the solubility is dissolution limited, one would expect a higher solubility by starting with salts versus salt-free. The salt effect is expected to be more pronounced using solid method compared to Dried-DMSO. For example, for AZ10, the Dried-DMSO value of citric salt and free-base are essentially the same (entry 10, Table 3), but the solid value of citric salt (198 μM) is much higher than the free-base (79 μM). The multiple batch data shown in Fig. 4, where some of the batches are salts, are also consistent with small salt effect when using the Dried-DMSO method.

3.12. Solubility and polymorphism considerations

Polymorphism issues, including metastable polymorphs, the existence of multiple stable polymorphs, or hydrate versus anhydrous forms, could present challenges in formulation design and other considerations in advancing development candidates. Their complete understanding would require extensive solid-state characterization normally carried out in later stage of discovery process or in early development. Such extensive characterization is not feasible in discovery phase due to large number and batches of compounds synthesized. It is conceivable that a project could benefit if potential polymorph issues are identified early on. Limited data collected in the present study suggests that large differences of solubility data collected using both Dried-DMSO and solid method could provide some indication of these potential issues. Representative data are summarized in entry 11–13, Table 3. AZ11 is an example of a compound that could exist as either mono-hydrate or amorphous solid. The Dried-DMSO solubility of AZ11 is nearly 20 times higher than solid solubility. AZ12 is an example of a compound that could exist in multiple stable polymorphs. Solid-state characterization of batches 3 and 4 showed they are of different polymorph and both are stable. Again, its Dried-DMSO solubility is very different from solid solubility. AZ13 is an example of metastable polymorph. For batch 5 of AZ13, the solid solubility is lower than Dried-DMSO solubility. When batch 6 was made from lyophilization, presumably forming amorphous material, the solid solubility is similar to Dried-DMSO solubility. Batch 7 was made by dissolving batch 5 in solution followed by lyophilization; its solid solubility is similar to batch 6. AZ13 is a competitor compound in Phase II clinical trial. The company disclosed that the Phase II trial needed to be repeated due to formulation issues. Another disclosure several years later indicated the compound had been shelved.

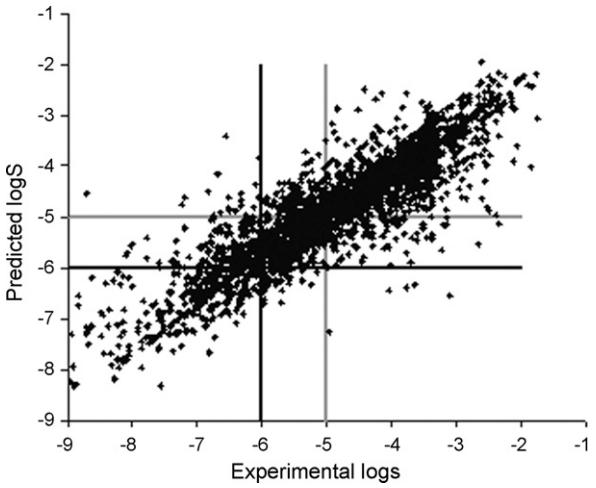


Fig. 6. Predicted versus measured log molar solubility of the test set compounds (black and gray lines marking 1 μM and 10 μM solubility, respectively).

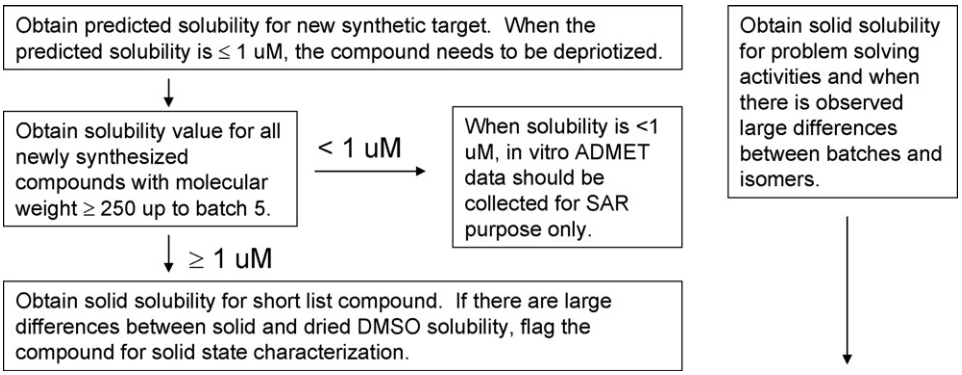
Based on these observations, the large differences of Dried-DMSO and solid solubility are considered to be undesirable and projects are advised to engage formulation support early on to have more detailed understanding of solid-state properties.

3.13. Predictive solubility for synthetic design

Review of historical data revealed that for some projects especially those targeting G Protein Coupled Receptors, the percentage of *in vitro* active compounds having solubility <1 μM could be as high as 38%. It is apparent that if these poorly soluble compounds were not synthesized in the first place, the implication on increased productivity and potential reduced rate of attrition could be enormous. Toward this goal, a solubility prediction model was developed in 2005. A plot of predicted versus the measured solubility of the 3730 test set compounds covering many discovery projects is shown in Fig. 6. Considering that measured solubility could be affected by many factors as discussed in previous sections, the performance of the prediction model is considered reasonable.

Although the model may not have enough accuracy for solubility prediction across all solubility ranges, we found it is extremely accurate in predicting poorly soluble compounds. As shown in Fig. 6, of 421 of the test set compounds having predicted solubility <1 μM, 376 compounds indeed have measured solubility lower than 1 μM, and another 39 compounds having measured solubility between 1 μM and 10 μM, i.e. an overall 98.6% compound that having measured solubility <10 μM. The model also predicts 1450 of the test set compounds have solubility lower than 10 μM. The measured solubilities of 1292 of these compounds are indeed lower than 10 μM.

It was noted before that in many cases QSAR model performance deteriorates away from its training space. Due to closing of old discovery projects and starting of new ones, the chemistry space in which our medicinal chemists hunt for drug candidates changes over time. To examine time-dependent performance of the prediction model, results of solubility measurements from March 1, 2006 to May 1, 2008 were retrieved. There are a total of 5664 compounds with numerical solubility data measured in this time period. The model predicted 274 of the compounds have solubilities lower than 1 μM. Among the 274 compounds, 218 have measured solubility lower than 1 μM. In addition, measured solubilities of an additional 47 compounds are between 1 μM and 10 μM. That is, if the model predicts a compound to have solubility lower than 1 μM, there is an 80% chance the measured solubility is indeed



Scheme 3. Solubility process recommendation to support CNS discovery.

Table 4
Percent of poorly soluble compounds synthesized at AZ Wilmington.

Year	2005	2006	2007	2008
% Compounds with Solubility <1 μM	19.5	12.3	13.7	7.4
% Compounds with Solubility <10 μM	39	37.1	34.2	25.1

lower than 1 μM and a 97% chance the measured solubility is lower than 10 μM.

Based on performance of the prediction model, projects were recommended in 2006 to deprioritize compounds having poorly predicted solubility from synthesis. Since then and with the predictive model as part of overall strategy for compound design, we have seen significant reduction in the percentage of poorly soluble compounds synthesized. Table 4 gives a summary of poorly soluble compounds synthesized at AstraZeneca Wilmington site over the past few years. It shows that before the prediction model was used for prioritizing synthesis targets (2005), compounds synthesized with solubility below 1 μM and 10 μM are as high as 19.5% and 39.0%, respectively. Since introduction of the prediction model in the second half of 2006, we have seen steady decrease in the percentage of poorly soluble compounds synthesized, achieving a 7.4% for those with solubility less than 1 μM and 25.1% with solubility less than 10 μM in the first half of 2008.

4. Conclusions and recommendations

A rapid throughput Dried-DMSO equilibrium solubility method is described. The method utilizes DMSO stock solution that is prepared and stored in central liquid dispensary, which is shared by physical properties and other assays such as *in vitro* biology and *in vitro* ADME screenings. In this way, solubility measurement uses only a fraction of a milligram, a saving of greater than 80% compared to traditional solubility measurement using solid material where a minimum of 2 mg is needed. The method is fully automated in sample preparation, data acquisition, and data processing. The range of measured solubility is from nM to an upper limit of 500 μM, sufficient for ranking project compounds. When a higher solubility limit is desired, one can simply start with more volume of DMSO stock solution. The method has been used successfully to support all projects including both early and late phases. The in-house solubility predictive model, based on the vast data set collected has helped projects to reduce the number of poorly soluble compounds synthesized by more than 30%.

The Dried-DMSO method offers rapid throughput capacity and has been used as the primary solubility method. The lower throughput solid method is reserved for problem solving and profiling of shortlist compounds. Extensive validation using both methods suggests good correlation for the majority of compounds. Since the solubility value of a given compound could be affected by many factors including solid-state properties, impurity and crystallinity, measurement of multiple batches is optimum to provide quality data and offer effective problem solving. Analysis of project compounds suggests that Dried-DMSO solubility data are more consistent from batch-to-batch and generally independent of salt forms compared to solid solubility. Available data also suggest that when 24 h equilibration is not reach, Dried-DMSO data could be complementary to solid value by providing solubility from a solid-state that is different from initial dry powder. This information could be valuable in providing early warnings on potential polymorph issues of the compound in question. These and other observations have formulated our general solubility strategy to support CNS discovery as shown in Scheme 3.

The process starts with requiring projects to employ in-house predictive model in synthetic design. When the predicted value is less than 1 μM, synthesis of the compound should be deprioritized. Once the compound is synthesized, solubility will be measured for all compounds with MW ≥ 250 and up to batch 5 using the Dried-DMSO method. When the measured value is less than 1 μM, collection of *in vitro* ADMET and *in vivo* data are recommended for SAR purposes only, especially for resource intensive assays. Once a compound has reached shortlist stage, collection of solubility using Solid method is recommended to confirm solubility and explore potential polymorph issues. Solid method is also used for problem solving activities throughout the process. Note that this 1 μM value in the recommendation can be viewed as a conservative value for general project applications. Project specific higher cutoff for both design and advancement, based on chemical space and project specific predictive model, has also been successfully employed.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2009.02.007.

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